[4] *R. Hagen, E. Heilbronner, W. Meier & P. Seiler,* Helv. *50,* 1523 (1967).
[5] *A. V. Willi,* «Säurekatalytische Reaktionen der organischen Chemie», S. 45, Vieweg, Braunschweig 1965.
[6] *B. C. Challis & F. A. Long,* J. Amer. chem. Soc. *87,* 1196 (1965).
[7] *A. J. Kresge, L. E. Hakka, S. Mylonakis & Y. Sato,* Disc. Faraday Soc. *39,* 75 (1965).
[8] *R. H. Boyd,* J. Amer. chem. Soc. *85,* 1555 (1963).
[9] *E. Heilbronner & S. Weber,* Helv. *32,* 1513 (1949); *J. E. B. Randles & J. M. Tedder,* J. Chem. Soc. *1955,* 1218; *K. N. Bascombe & R. P. Bell, ibid. 1959,* 1095.
[10] *K. H. Grellmann, E. Heilbronner, P. Seiler & A. Weller,* J. Amer. chem. Soc. *90,* 4238 (1968).

294. Structure of Brefeldin A

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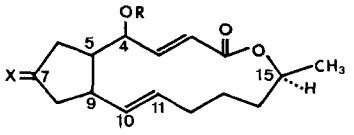
(11. XI. 71)

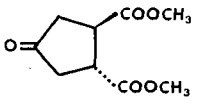
Summary. On the basis of *X*-ray crystal structure analysis, CD. measurements, and asymmetric synthesis the absolute configuration of brefeldin A has been determined.

Some years ago we reported the isolation [1] and the elucidation of the constitution [2] of brefeldin A (**1**) (identical with ascotoxin [3], decumbin [4] and cyanein [5]). At that time the (*S*)-chirality of C15, the *trans* connection of the two rings and the *trans* relationship at the conjugated double bond had been established, but the stereochemistry of the other asymmetric centers and of the isolated double bond remained to be clarified.

A recent publication by *Suzuki et al.* [3] prompts us to describe our own results concerning relative and absolute configuration of brefeldin A, which are not fully in agreement with those of the Japanese authors.



1 R = H X = H, OH **2** R = Ac X = H, OH
3 R = Ac X = O

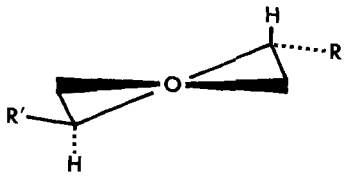


4

The NMR. spectrum of brefeldin A (**1**) ($SO(CD_3)_2$-$CDCl_3$ 7:3, 100MHz) shows signals for the protons on the isolated double bond at 5.22 and 5.60ppm (tetramethylsilane = 0) with a coupling constant of 15.5 cps, which indicates their *trans* relationship. The method of *Horeau* [6] allowed[1]) to specify the chirality at C4 as (*S*) in brefeldin A (**1**) and (*R*) in tetrahydro brefeldin A [1].

[1]) The resulting α-phenyl butyric acid had $\alpha = -0.091°$ in the case of brefeldin A and $\alpha = +0.087°$ in the case of tetrahydro-brefeldin A. The optical yield in both cases was 23%. Owing to its symmetrical position the hydroxyl group in the five-membered ring appears to be without influence on the asymmetric synthesis.

A negative *Cotton* effect[2]) was found with 4-O-acetyl-7-oxo brefeldin A (**3**, m.p. 123–124°, M^+ 320). The latter was obtained by *Jones* oxidation [7] of **2**(m.p. 128–129°, M^+ 322) prepared by reacting **1** with one equivalent of Ac_2O in pyridine. On the basis of this evidence the chirality of the five-membered ring is as follows [8]:



Assuming that the bulky groups at C5 and C9 are in the more stable equatorial positions [8], the chiralities of C5 and C9 in brefeldin A (**1**) can be specified as (*R*) and (*S*) respectively. The absolute configuration of dimethyl (–)-cyclopentanone-*trans*-3,4-dicarboxylate (**4**), a degradation product [2] of brefeldin A (**1**), is thus also defined, *i.e.* (*R*) for both asymmetric centers. As would be expected, therefore, **4** shows a negative *Cotton* effect[3]).

To confirm these findings and to elucidate the configuration at C7, brefeldin A (**1**) was submitted to an *X*-ray crystal structure analysis (Fig. 1). The absolute configuration of the molecule follows from the (*S*)-chirality [2] of C15 and is in agreement with the predictions based on asymmetric synthesis [6] and CD. measurements.

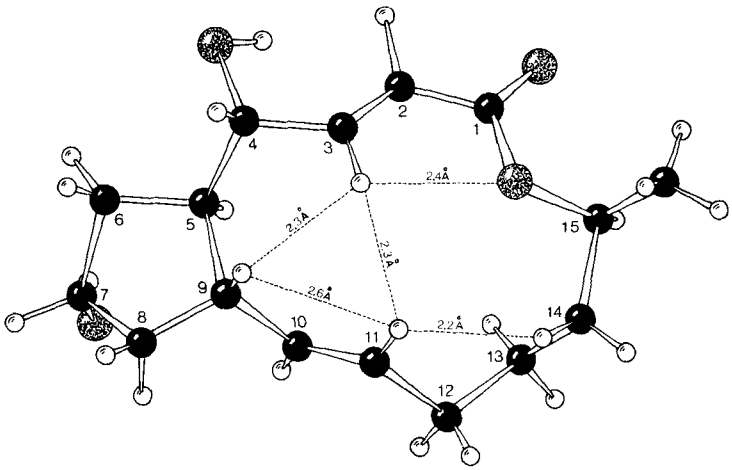


Fig. 1. *A perspective view of brefeldin A*
● = carbon, ◉ = oxygen, ○ = hydrogen

Crystallographic Data: brefeldin A, $C_{16}H_{24}O_4$, crystallises in colourless prismatic needles from methanol/acetone. Cell dimensions and space group were determined from precession photographs. Intensity data were collected on a linear diffractometer [9] with graphite monochromatised Mo$K\alpha$ radiation. The experimental results are summarized as follows.

[2]) CD. maxima (in ethanol): 321 (–.60), 308 (–2.54), 296 (–3.94), 289 (–3.91), 259 (–1.76), 215 (+13.2), 206 (+17.0).

[3]) CD. maxima (in CH_3CN): 312 (–.92), 302 (–1.85), 293 (–2.03), 284 (–1.65), 217 (–.99).

Cell dimensions: $a = 18.83(2)$ Å; $b = 7.37(1)$; $c = 10.99(1)$. Volume: $V = 1524$ Å^3. Space Group: $P2_12_12_1$. Density, observed $1.22\,g \cdot cm^{-3}$, calculated $1.21\,g \cdot cm^{-3}$. Total number of reflexions ($\sin\theta/\lambda < \cdot 56$ Å^{-1}): 1342. Number of significant reflexions ($I > 3\sqrt{(P+B)}$): 1024. $\langle|E|\rangle = .844$; $\langle|E^2-1|\rangle = .807$; $\langle E^2\rangle = .976$; B (overall) = 3.6 Å^2.

Structure analysis. The structure was solved by a method similar to the 'multi-solution method' described by *Germain, Main & Woolfson* [10]. The hydrogen positions were determined from a difference *Fourier* map after preliminary refinement of the C- and O-atoms. A final refinement by block diagonal least squares, including all observed reflections in the calculations, concluded the analysis with an R-value of 0.047. A list of structure factors will be sent to interested parties upon request (*HPW*). Coordinates are given in the Table. Anisotropic vibrational parameters for C- and O-atoms and isotropic values for H-atoms are available upon request.

Fractional coordinates of brefeldin A

	x	y	z		x	y	z
C1	.2946	.2906	.4213	H[C4]	.530	.262	.459
C2	.3632	.3034	.4866	H[C5]	.460	-.058	.594
C3	.4218	.2291	.4441	H1[C6]	.577	-.005	.687
C4	.4902	.2039	.5124	H2[C6]	.618	.018	.567
C5	.5017	.0001	.5361	H[C7]	.630	-.319	.599
C6	.5715	-.0396	.6032	H1[C8]	.614	-.236	.392
C7	.5841	-.2385	.5754	H2[C8]	.561	-.355	.423
C8	.5678	-.2497	.4405	H[C9]	.523	-.023	.349
C9	.5076	-.1173	.4154	H[C10]	.427	-.310	.442
C10	.4401	-.2076	.3820	H[C11]	.408	-.068	.225
C11	.4030	-.1831	.2824	H1[C12]	.337	-.398	.298
C12	.3354	-.2860	.2504	H2[C12]	.338	-.326	.164
C13	.2671	-.1789	.2738	H1[C13]	.273	-.157	.359
C14	.2539	-.0194	.1879	H2[C13]	.227	-.268	.277
C15	.2346	.1580	.2512	H1[C14]	.312	-.018	.125
O16	.2998	.2059	.3151	H2[C14]	.223	-.060	.131
C16	.2122	.3045	.1669	H[C15]	.192	.123	.316
O[C1]	.2403	.3501	.4614	H1[C16]	.247	.328	.112
O[C4]	.4906	.2927	.6279	H2[C16]	.178	.285	.103
O[C7]	.5343	-.3536	.6395	H3[C16]	.194	.399	.200
				H[O(C4)]	.448	.303	.625
H[C2]	.359	.395	.571	H[O(C7)]	.528	-.308	.715
H[C3]	.423	.164	.359				

The molecular parameters are summarized in Fig. 2 and 3. None of the values for bond lenghts and angles deviate more than 3σ from accepted values. The α,β-unsaturated lactone group is planar, but the bond lengths of C1–C2, 1.48(1) Å, and of C2=C3, 1.32(1) Å, indicate no conjugation in the system. It is interesting to note that the *trans*-relationships of the vicinal hydrogens on C4–C5 and on C9–C10 seem to be retained in solution as is indicated by their NMR. coupling constants of about 9 Hz. The five-membered ring has approximately C_2-symmetry with the twofold axis going through C9 and the middle of C6–C7. There are no intramolecular hydrogen bonds and none of the transannular distances in the macrocycle are unexpectedly short (Fig. 1).

An inspection of the molecular packing in the crystal revealed neither hydrogen bonds nor any other close intermolecular contacts.

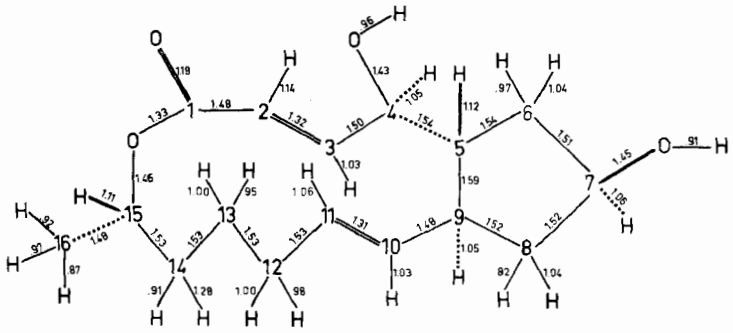


Fig. 2. *Bond lengths in brefeldin A*
The average ESD. of C—C and C—O bonds is 0.008 Å, for C—H and O—H bonds 0.08 Å

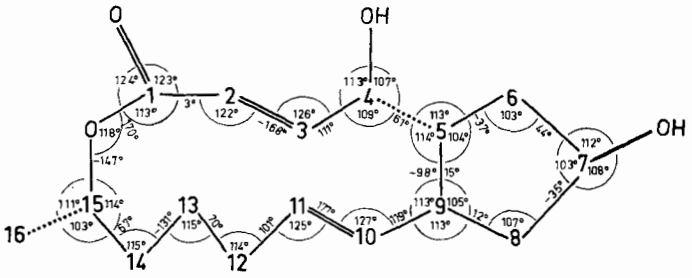


Fig. 3. *Bond angles and torsion angles in brefeldin A*
The average ESD. for bond angles is about 0.8°, for torsion angles about 1.0°

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BIBLIOGRAPHY

[1] *E. Haerri, W. Loeffler, H. P. Sigg, H. Stachelin & Ch. Tamm*, Helv. *46*, 1235 (1963).
[2] *H. P. Sigg*, Helv. *47*, 1401 (1964).
[3] *Y. Suzuki, H. Tanaka, H. Aoki & T. Tamura*, Agr. biol. Chemistry *34*, 395 (1970).
[4] *V. L. Singleton, N. Bohonos & A. J. Ullstrup*, Nature *181*, 1072 (1958).
[5] *V. Betina, P. Nemec, J. Dobias & Z. Barath*, Folia microbiol. *7*, 353 (1962).
[6] *A. Horeau*, Tetrahedron Letters *1961*, 506, 654; *1962*, 965.
[7] *K. Bowden, I. M. Heilbron, E. R. H. Jones & B. C. L. Weedon*, J. chem. Soc. *1946*, 39.
[8] *W. Klyne*, Tetrahedron *13*, 29 (1961); *P. Crabbe*, in 'Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry', edited by *G. Snatzke*, Heyden and Son, Ltd., London 1967; *O. Korver*, Rec. Trav. chim. Pays-Bas *88*, 1070 (1969); *C. Ouannes & J. J. Jacques*, Bull. Soc. chim. France *1965*, 3611.
[9] *U. W. Arndt & C. D. Phillips*, Acta cryst. *14*, 807 (1961).
[10] *G. Germain, P. Main & M. M. Woolfson*, Acta cryst. *B 26*, 274 (1970).